REVIEW ARTICLE

Polar Body Diagnosis – A Step in the Right Direction?

Katrin van der Ven, Markus Montag, Hans van der Ven

SUMMARY

Introduction: Polar body diagnosis (PBD) is a new diagnostic method for the indirect genetic analysis of oocytes, which is carried out as part of in vitro fertilization. The biopsy of polar bodies is technically demanding and cannot be adopted uncritically in routine practice, in the absence of robust data to support this laboratory procedure.

Methods: Selective literature review and analysis of own PBD data.

Results: The main application of PBD is the detection of chromosomal aneuploidies and maternally inherited translocations in oocytes. The major disadvantage of PBD is that the paternal contribution to the genetic constitution of the developing embryo cannot be evaluated. Moreover, the potential value of polar body biopsy for the diagnosis of monogenetic diseases is limited.

Discussion: The role of PBD in improving of success rates in assisted reproduction requires evaluation in further clinical trials. For maternal translocations, PBD can be used to reduce the risk of miscarriage. Rapid development in the field of molecular diagnostic and biopsy techniques will also influence PBD and will most likely allow wider application of this method in the near future.

Polar body diagnosis is a method for the genetic analysis of oocytes before the end of fertilization (preconception diagnosis) (1). Removal and analysis of the first and second polar body provides indirect evidence of the genetic constitution of the oocyte. In contrast, preimplantation diagnosis (PID) allows direct analysis of the genetic makeup of a developing embryo by extraction and examination of individual blastomeres (2). PBD and PID can only be performed as part of in vitro fertilization therapy. The procedures allow the demonstration of numerical chromosome maldistributions (aneuploidies), translocations, and monogenic diseases. These methods have the goal of improving the success rates of assisted reproduction and of preventing pregnancies that lead to the birth of severely ill children.

Because of its greater diagnostic value, preimplantation diagnosis has gained international acceptance. In Germany, PID is considered to be in contravention of the German Embryo Protection Act. Polar body diagnosis has therefore become established during the ongoing ethical and legal debate about the tenor and utility of this legislation.

In addition to presenting the methodological aspects, this article explains and discusses, on the basis of a selective review of the literature, the possible uses and the value of polar body diagnosis in various areas of diagnostic activity within the temporal and legal framework prescribed by the German Embryo Protection Act.

Meiosis with formation of the first and second polar body

The diploid chromosome set of the oocyte is reduced to a haploid chromosome set shortly before ovulation by completion of the first meiosis *(figure 1)*. One set of chromosomes remains in the oocyte, while the second chromosome set is expelled from the cytoplasm with formation of the first polar body.

The penetration of a sperm is followed by the second meiosis. The doublethreaded chromosomes divide further into chromatids and one chromatid set is expelled with formation of the second polar body. The number of chromosomes and chromatids in polar bodies is the same after normally completed first and

Abteilung für Gynäkologische Endokrinologie und Reproduktionsmedizin, Zentrum für Geburtshilfe und Frauenheilkunde, Universitätsklinikum Bonn: Prof. Dr. med. van der Ven, PD Dr. rer. nat. Montag, Prof. Dr. med. van der Ven

second meiosis. The polar bodies are of no proven significance for further embryonic development and are available for diagnostic purposes.

After fertilization, the male and female pronucleus containing the maternal and paternal genomes develop in the oocyte. 16 to 20 hours later the pronuclear membranes break down in preparation for the first mitosis. In biological terms, the fertilization process is now complete.

Provisions of the German Embryo Protection Act

The German Embryo Protection Act of 1990 defines the temporal and therapeutic limits for artificial fertilization procedures in Germany.

In the German Embryo Protection Act the fertilized, viable human oocyte from the point of nuclear fusion onwards, as well as every totipotent cell taken from an embryo is already defined as an embryo. Embryos may only be produced for the purpose of embryo transfer. Based on the existing interpretation, not more than three oocytes may be fertilized and not more than three embryos transferred to the mother at one time. Since polar body biopsy takes place before the fusion of the pronuclei, it is a preconception diagnostic technique and is compatible with the German Embryo Protection Act. However, only a limited time frame of a maximum 20 hours between penetration of the sperm and appearance of the pronuclei is available to perform PBD *(figure 2)*.

The narrow time limits specified by the German Embryo Protection Act could theoretically be extended by cryopreserving the oocytes at the pronuclear stage until completion of the genetic diagnostic program. Despite the good survival and development rates of cryopreserved oocytes following polar body biopsy, so far only a small number of continuous clinically detectable pregnancies have been achieved. Because of unresolved cryobiological problems, this procedure is not yet an acceptable strategy at present.

Occurrence and incidence of aneuploidies

Aneuploidies, i.e. deviations from the regular number of chromosomes, are predominantly the result of maldistributions of chromosomes during meiosis *(figure 3)*. Up to 80% of aneuploidies occur during the first meiosis. The frequency of aneuploidies in oocytes increases sharply after the age of 35 years. In a 40-year-old woman, an estimated 50% to 70% of the mature oocytes are affected by a chromosomal abnormality (3).

This explains the rising risk of abortion in patients of high maternal age. Natural loss of embryos with an abnormal set of chromosomes already starts in the early stages of embryonic development and is regarded as one of the factors responsible for the comparatively low fertility of humans (4). However, recent data have also revealed considerable interindividual differences in the number of chromosomally abnormal oocytes and embryos in young women (5, 6, 7). More than

TABLE 1

Polar body diagnosis (PBD) versus preimplantation diagnosis (PID)

Polar body diagnosis	Preimplantation diagnosis
Advantages	**Advantages**
Polar bodies are not embryonic cells	Maternal and paternal genome assessable
Polar bodies are dispensable for further embryonic development	Broader range of indications
	Possibly greater diagnostic reliability due to screening of several cells
Disadvantages	**Disadvantages**
Information only about maternal genome	Removal of embryonic cells
Restricted diagnostic spectrum	Possible restriction of embryonic development potential due to blastomere biopsy

90% of embryonic chromosome abnormalities are of maternal origin.

The aneuploidy rates in sperms are to be regarded as low in the presence of a normal paternal karyotype (1% to 2.5%), but rise significantly with increasing impairment of sperm quality. Nevertheless, sperms with an abnormal chromosomal constitution contribute only slightly to the risk of embryonic aneuploidy in artificial fertilization procedures (8).

Indications for polar body biopsy

In Germany, polar body diagnosis is performed mainly to improve therapeutic success rates in assisted reproduction and only in individual cases for the specific diagnosis of monogenic diseases (9, 10) or maternal translocations (11).

In assisted reproduction, the identification of chromosomally normal oocytes by polar body diagnosis should allow higher implantation and birth rates.

This could be beneficial especially for patients expected to have elevated rates of aneuploid oocytes. This is the case, for example, in older patients or in maternal translocations and possibly also when an implantation repeatedly fails after embryo transfer ("implantation failure") or in recurrent spontaneous abortions of undetermined etiology.

Technical laboratory requirements and diagnostic safety

Critical technical laboratory aspects of polar body diagnosis are:
- Atraumatic opening of the extracellular coat of the oocyte (zona pellucida)
- Correctly timed removal of the complete polar body
- Precise and comprehensive genetic diagnosis.

The rate of oocyte trauma following laser-mediated opening of the zona pellucida is reported as approximately 0.5% to 1% (12). Essential requirements,
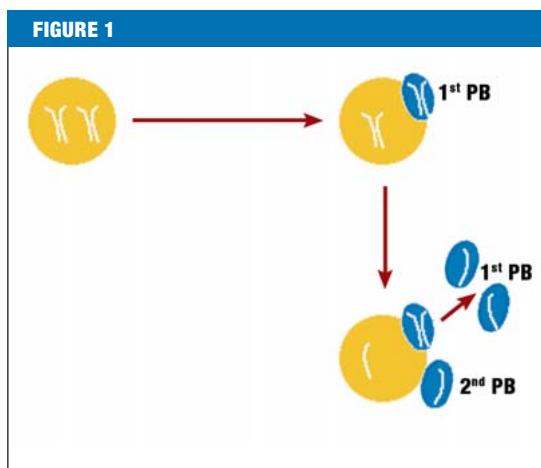
Meiosis with formation of the first and second polar body (PB)

however, remain an extensive laboratory routine and technical experience on the part of the biologists and geneticists involved. In aneuploidy diagnostics the theoretical benefit of PBD can only come statistically and clinically into effect when technical risks inherent in the methodology, such as oocyte trauma and misdiagnoses, are minimized.

It has been observed in preimplantation diagnostics that the extraction of one or two blastomeres can result in a significant reduction in the implantation

Time course of polar body diagnosis; NPB, nucleolus precursor bodies

potential of the embryos (13, 14, 15). The methodological procedure must be critically appraised in terms of the polarity of the early embryo because all blastomeres already exhibit differentiation markers at the four-cell stage (16). The removal of individual blastomeres could influence the polarity of the embryo and thus its further development potential, even though the remaining cells of the preimplantation embryo are postulated to have a certain compensatory plasticity. In PBD only polar bodies are taken that have no physiological significance for further embryonic development. Whether PBD is superior to PID in aneuploidy diagnostics for this reason will have to be determined in further research.

## Methods for detection of chromosome maldistributions

After biopsy of the first and second polar body, different chromosomes (usually chromosomes 13, 16, 18, 21, 22) are detected by multisample fluorescence in situ hybridization (FISH). Meiotic maldistributions of these chromosomes are a frequent cause of monosomies and trisomies in clinically detectable pregnancies and result in miscarriages in a high percentage of cases. In the fluorescence microscopic evaluation it is determined how many copies of the investigated chromosomes/chromatids are present in the first and second polar body.

The result allows an indirect conclusion to be drawn regarding the chromosome set of the oocyte. With the existing FISH technique up to six chromosomes can be detected in one assay (17). Within the time frame prescribed by the German Embryo Protection Act, at most two sets of determinations with analysis of altogether 10 to 12 chromosomes can be performed, which considerably limits the scope of this method. The information value of the method is further restricted by the "FISH drop-out," in which one FISH probe fails to detect the chromosome to be analyzed although it is actually present. The frequency of FISH drop-out is estimated as 2% to 3% per investigated chromosome.

Further methods for simultaneous detection of all chromosomes have already been tested for this application, but are either not practicable for technical reasons (18, 19) or, despite recent technical advances, cannot yet be implemented within the time limits prescribed by the German Embryo Protection Act (e.g. comparative genomic hybridization [CGH] or chip technology) (20–24).

Diagnosis of monogenic diseases

Polar body diagnosis can be used for the investigation of monogenic diseases, but is clearly inferior to preimplantation diagnosis in practicability and diagnostic reliability. Physiological processes during meiosis – such as the exchange of genetic material between the homologous chromosomes in the prophase of the first meiosis (crossing over), possibly combined with premature chromatid segregation – reduce the
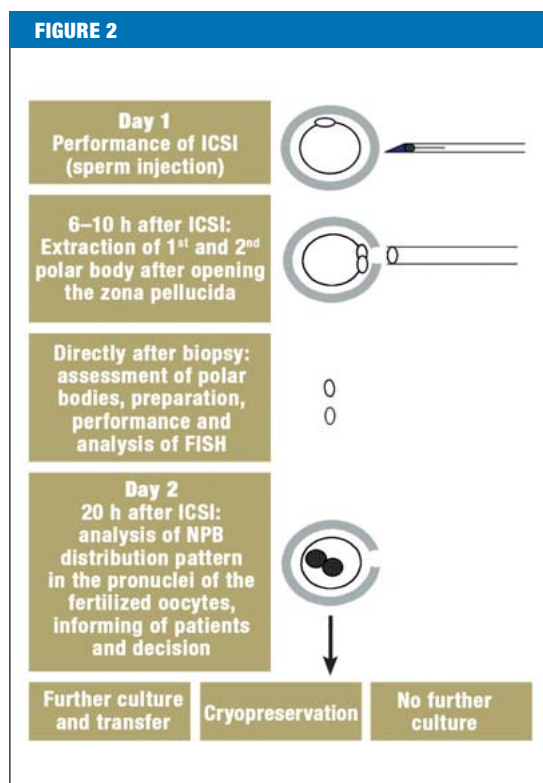
evidential value of this method and make an analysis of the first and second polar body indispensable to assure a correct diagnosis.

In monogenic diseases, the presence of the disease-specific mutation is demonstrated by a polymerase chain reaction (PCR). Besides the risk of contamination associated with single cell PCR, problems inherent in the method, such as the exclusive amplification of one of the disease alleles to be investigated (allele drop-out) must be taken into account. An amplification failure occurs in 10% to 20% of cases in single cell PCRs (25) and, if unrecognized, can lead to misdiagnosis.

One basic disadvantage of polar body diagnosis consists in the fact that only the maternal genome can be investigated and no statement can be made regarding possible paternal factors. This would be acceptable for maternal autosomal dominant or X-chromosome diseases of maternal origin, because all oocytes bearing mutations result in a sick child regardless of the genetic constitution of the father.

All oocytes bearing mutations (statistically 50%) also have to be discarded in the diagnosis of recessive inheritance, although the disease would only manifest in 25% of the resulting embryos. This is because only 50% of the sperms also bear the disease predisposition.

Predictive value of polar body biopsy in aneuploidy diagnosis

Estimates of the diagnostic reliability of polar body diagnosis are based on data obtained from aborted material. Assuming chromosome-specific trisomy rates in the aborted material it was estimated that approximately 50% of the chromosome aberrations occurring in the first trimester of miscarriages would be detected

FIGURE 3
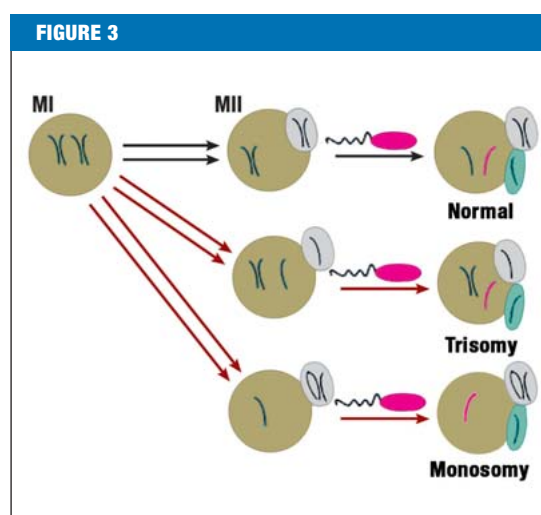
Development of numerical chromosome aberrations (trisomy/monosomy) on formation of the first polar body

by analyzing chromosomes 13, 16, 18, 21, and 22 (e1). The small number of chromosomes identifiable by polar body biopsy with FISH is thus a clear limitation of the method.

Comparative studies of oocytes with the associated first polar bodies by FISH and CGH showed that only 37% of the actually present chromosome abnormalities were detected using five FISH probes. When using 12 FISH probes, the detection rate increases to 67% of the aneuploidies of the oocyte/polar body pairs diagnosable by CGH (13, 22, 23).

Additional analysis of the second polar body markedly improves the detection rates of chromosome maldistributions. A series of analyses in 10 317 oocytes

TABLE 2

Results of PBD for aneuploidy screening in women between 35 and 39 years of age and with at least 2 previous IVF attempts

	PBD group	Control	Statistics
Treatment cycles	159	163	
Median age	37.8	36.9	n.s.
Transfer rate	89.3 % (142/159)	90.2 % (147/163)	n.s.
Embryos/transfer	1.77 (251/142)	2.02 (297/147)	$p < 0.05$
Biochemical pregnancy rate/transfer	31.7 % (45/142)	31.9 % (47/147)	n.s.
Clinical pregnancy rate/transfer	28.9 % (41/142)	21.8 % (32/147)	n.s.
Implantation rate	17.5 % (44/251)	11.8 % (35/297)	$p < 0.05$
Abortion rate	19.5 % (8/41)	28.1 % (9/32)	n.s.
Birth rate/cycle	20.8 % (33/159)	14.1 % (23/163)	n.s.
Birth rate/transfer	23.2 % (33/142)	15.6 % (23/147)	$p = 0.1$

ANOVA and chi-square test were used for statistical analysis;
PBD, polar body diagnosis; IVF, in vitro fertilization; n.s., not significant

TABLE 3

Results of PBD for aneuploidy screening in women aged ≥ 40 years

	PBD group	Control	Statistics
Treatment cycles	103	110	
Transfer rate	80.6 % (83/103)	92.7 % (102/110)	n.s.
Embryos/transfer	1.75 (145/83)	2.03 (207/102)	$p < 0.05$
Biochemical pregnancy rate/transfer	20.5 % (17/83)	18.6 % (19/102)	n.s.
Clinical pregnancy rate/transfer	14.5 % (12/83)	14.7 % (15/102)	n.s.
Implantation rate	9.7 % (14/145)	7.2 % (15/207)	n.s.
Abortion rate	14.3 % (2/14)	46.7 % (7/15)	$p = 0.06$
Birth rate/cycle	9.8 % (10/103)	7.3 % (8/110)	n.s.
Birth rate/transfer	12.0 % (10/83)	7.8 % (8/102)	n.s.

ANOVA and chi-square test were used for statistical analysis;
PBD, polar body diagnosis

from 1551 IVF cycles (e2) with FISH analysis of the first and second polar body found an aneupoloidy rate of 61.8% for five chromosomes (chromosomes 13, 16, 18, 21, 22). One third of the aneuploidies discovered developed during the second meiosis and were therefore only detectable in the second polar body (6, e2, e3).

While the aneuploidy rates in oocytes increase with maternal age (6, e3), postmeiotic abnormalities that develop during the mitotic divisions of the early embryo (e.g., chromosome mosaics) show similar incidences in all age groups.

Postmeiotic chromosome abnormalities, but not aneuploidies, correlate clearly with changed morphology and reduced mitotic rate of the affected embryos (e3). Since the German Embryo Protection Act requires the selection of oocytes for later embryo transfer to be performed at the pronuclear stage, the assessment criteria for embryo quality mentioned above cannot be used in Germany.

Recent research has shown that the karyotype of the mature oocyte is the main factor determining the developmental potential of the resulting embryos. The majority of euploid oocytes develop into euploid embryos, a much higher percentage of which in turn reach the blastomere stage than embryos with chromosome abnormalities (93% versus 21%) (e4). Particularly in Germany, aneuploidy diagnosis is thus an important tool for identifying oocytes with high developmental potential.

While chromosome abnormalities originating in meiosis affect all cells of the embryo, postmeiotic aneuploidies may be heterogeneous in terms of the number of blastomeres affected and the effects on the further development of the embryo.

The high discordance of the chromosome findings obtained in PID in different blastomeres of the same embryo (7, e5), and the definition of adequate consequences in the event of abnormal findings currently represent a significant practical problem in preimplantation diagnosis (e5, e6).

Results of PBD for aneuploidy screening

As in preimplantation diagnostics (12, 15, e6, e7), the value of aneuploidy screening in polar body biopsy is currently disputed as a means of increasing the success rates of extracorporeal fertilization.

On the international scale, polar body diagnosis for detection of aneuploidy is only used extensively by the research team of Verlinsky. The most comprehensive retrospective results documentation of this group covers more than 1200 treatment cycles in patients with an average age of 38.5 years and a "poor prognosis" not defined more closely in reproductive medical terms. The clinical pregnancy rate of all cycles with embryo transfer after analysis of five chromosomes was reported as 22%. On average, 2.35 embryos were transferred (e8). No control group is presented.

The German IVF registry (Deutsches IVF Register, DIR), which prospectively records all the IVF cycles performed in Germany, shows a clinical pregnancy rate per embryo transfer of 21.3% (DIR 2003) for all patients over 35 years after regular IVF without polar body diagnosis. An evaluation of PBD in 460 women from a German IVF center revealed, as stated in the publication of Verlinsky (e6), clinical pregnancy rates which in the absence of a control group were even below the comparative data of DIR (e9).

Controlled studies or at least the inclusion of a center-related control group is therefore essential to demonstrate the effective benefit of PBD for aneuploidy diagnosis. In compliance with this requirement, after optimizing the laboratory techniques the authors have evaluated their own treatment data prospectively documented in a DIR-compatible recording program according to cycles with and without

PBD. The results for the subgroup of women aged 35 to 39 years with at least two previous IVF/ICSI treatment trials are shown in *table 2*. These data show that, despite a smaller number of transferred embryos, significantly higher implantation rates were achieved in the PBD group.

An evaluation for patients over 39 years of age reveals that after performing PBD the rate of abortions decreases with a comparable clinical pregnancy rate *(table 3)*. These results suggest that an indication-based use of PBD could certainly provide benefits, for example in older patients. Further studies in larger patient populations and under standardized laboratory conditions, however, will be essential for the clinical and scientific evaluation of PBD. One initiated multicenter study could not be continued because of differences in laboratory routine and biopsy techniques.

Prospects

Polar body diagnosis is a new method for the indirect genetic screening of the oocyte, and its therapeutic benefits in specific patient populations still require to be conclusively proven. Although already established in several German laboratories, polar body diagnosis is technically demanding and not a routine method that should not be adopted uncritically in routine practice.

Concurrently with the clinical evaluation and definition of clear indication groups, a further optimization of the laboratory techniques is desirable. This includes improving the biopsy techniques and the cryopreservation of oocytes after polar body biopsy and increasing the number of investigated relevant chromosomes in aneuploidy diagnosis. It is generally accepted that the further development of molecular genetic methods will also influence the clinical significance of polar body diagnosis in the years ahead.

A basic disadvantage of polar body diagnosis compared to preimplantation diagnosis by blastomere biopsy will continue to be that paternal factors are not diagnosable and monogenic diseases are only diagnosable to a limited extent. It should also be emphasized that polar body biopsy is not an earlier form of prenatal diagnosis and cannot replace this procedure.

In awareness of these limitations, polar body diagnosis, for example for aneuploidy screening, is nevertheless a step in the right direction as an indication-based supporting procedure for sterility therapy under the limited conditions imposed by the German Embryo Protection Act.

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Corresponding author
Prof. Dr. med. Hans van der Ven
Abteilung für Gynäkologische Endokrinologie und Reproduktionsmedizin
Zentrum für Geburtshilfe und Frauenheilkunde
Universitätsklinikum Bonn
Sigmund-Freud-Str. 25, 53105 Bonn, Germany

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